Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Michael Trauner, Aliya Gulamhusein, Bilal Hameed, et al.

The nonsteroidal FXR agonist cilofexor (GS-9674) improves markers of cholestasis and liver injury in patients with PSC

Table of Contents

Eligibility Criteria

Inclusion Criteria

Patients must meet all of the following inclusion criteria to be eligible for participation in this study.

1) Males and females between 18-70 years of age; inclusive based on the date of the screening visit;
2) Willing and able to give informed consent prior to any study specific procedures being performed;
3) Diagnosis of PSC based on cholangiogram (magnetic resonance cholangiopancreatography [MRCP], endoscopic retrograde cholangiopancreatography [ERCP], or percutaneous transhepatic cholangiogram [PTC]) within the previous 12 months;
4) Serum ALP > 1.67 x ULN;
5) For patients on UDCA, the dose of UDCA must have been stable for at least 12 months before screening through the end of the treatment. For patients not on UDCA, no UDCA use for at least 12 month before screening through the end of the treatment;
6) For patients being administered biologic treatments (eg, anti-tumor necrosis factor (TNF) or anti-integrin monoclonal antibodies), immunosuppressants or systemic corticosteroids, the dose must have been stable for at least 3 months prior to screening and anticipated to remain stable throughout the trial;
7) Screening FibroSURE/FibroTest® < 0.75, unless a historical liver biopsy within 12 months of screening does not reveal cirrhosis. In patients with Gilbert's syndrome or hemolysis, FibroSURE/FibroTest® will be calculated using direct bilirubin instead of total bilirubin.
8) Platelet count ≥ 150,000/mm3;
9) Albumin ≥ 3.3 g/dL;
10) Serum creatinine ≤ ULN
11) In patients with a history of IBD, a colonoscopy within 6 months of screening must demonstrate no evidence of active IBD;
12) Females of childbearing potential (as defined in Appendix 4) must have a negative serum pregnancy test at the Screening visit and a negative urine pregnancy test on the Baseline/Day 1 visit prior to the first dose of study drug;
13) All female patients of childbearing potential who engage in heterosexual intercourse must agree to use a highly effective method of contraception from the screening visit throughout the study period and for 30 days following the last dose of study drug(s) (see definition in Appendix 4);
14) Male patients with female partners of childbearing potential must use condoms during treatment and for 90 days after the last dose of study drug(s);
15) Male patients must agree to avoid sperm donation from Baseline/Day 1 visit throughout the study period and for 90 days after the last dose of study drug(s);
16) Female patients must refrain from egg donation and in-vitro fertilization during treatment and until at least 30 days after the last dose of study drug(s);
17) Willing and able to comply with scheduled visits, drug administration plan, laboratory tests, liver biopsies, other study procedures, and study restrictions;
18) Must be able to read and complete QoL questionnaires independently.

Exclusion Criteria

Patients who meet any of the following exclusion criteria are not to be enrolled in this study.

1) Pregnant or lactating females; lactating females must agree to discontinue nursing before the study drug (s) is administered;
2) ALT > 10x ULN;
3) Total bilirubin > 2x ULN;
4) INR > 1.2 unless on anticoagulant therapy;
5) Cirrhosis of the liver as defined by any of the following:
    a) Historical liver biopsy demonstrating stage 4 fibrosis (e.g. Ludwig stage 4 or Ishak stage ≥5)
    b) History of decompensated liver disease, including ascites, hepatic encephalopathy or variceal bleeding
    c) Liver stiffness >14.4 kPa by FibroScan;
6) Small-duct PSC (histologic evidence of PSC with normal bile ducts on cholangiography);
7) Other causes of liver disease including secondary sclerosing cholangitis and viral, metabolic, alcoholic, and other autoimmune conditions Patients with hepatic steatosis may be included if there is no evidence of nonalcoholic steatohepatitis (NASH) in the opinion of the investigator or on liver biopsy;
8) Positive anti-mitochondrial antibody;
9) History of liver transplantation;
10) History of hepatocellular carcinoma or cholangiocarcinoma. If a dominant stricture is found, cholangiocarcinoma must be excluded prior to randomization;
11) Ascending cholangitis within 60 days of screening;
12) Presence of a percutaneous drain or bile duct stent;
13) Chronic hepatitis B (HBsAg positive);
14) Chronic hepatitis C (HCV antibody and RNA positive);
15) HIV Ab positive;
16) Current active IBD defined as a partial Mayo score of > 1 and/or a score in the Rectal Bleeding domain > 0;
17) Known hypercoagulable condition or history of venous or arterial thromboembolic disease;
18) Alcohol consumption greater than 21 oz/week for males or 14 oz/week for females (1oz/30mL of alcohol is present in 1 12oz/360mL beer, 1 4oz/120mL glass of wine, and a 1 oz/30 mL measure of 40% proof alcohol);
19) History of intestinal resection or malabsorptive condition that may limit the absorption of GS-9674. Prior cholecystectomy and appendectomy are permitted;
20) Use of fibrates or obeticholic acid within 3 months prior to screening through the end of treatment;
21) Use of antibiotics (e.g., vancomycin, metronidazole, minocycline, etc.) for the treatment of PSC within 60 days of screening;
22) Positive urine screen for amphetamines, cocaine or opiates (i.e. heroin, morphine) at screening. Patients on stable methadone or buprenorphine maintenance treatment for at least 6 months prior to screening may be included in the study. Patients with a positive urine drug screen due to prescription opioid-based medication are eligible if the prescription and diagnosis are reviewed and approved by the investigator;

23) Unstable cardiovascular disease as defined by any of the following:
    a) Unstable angina within 6 months prior to screening
    b) Myocardial infarction, coronary artery bypass graft surgery or coronary angioplasty within 6 months prior to screening
    c) Transient ischemic attack or cerebrovascular accident within 6 months prior to screening
    d) Obstructive valvular heart disease or hypertrophic cardiomyopathy
    e) Congestive heart failure;
24) Use of any prohibited concomitant medications as described in Section 5.5;
25) History of a malignancy within 5 years of screening with the following exceptions:
a) Adequately treated carcinoma in situ of the cervix
b) Adequately treated basal or squamous cell cancer or other localized non-melanoma skin cancer;
26) Any laboratory abnormality or condition that, in the investigator's opinion, could adversely affect the safety of the patient or impair the assessment of study results;
27) Participation in another investigational study of a drug or device within 1 month prior or within 5 half-lives of the prior investigational agent (whichever is longer) prior to screening;
28) Concurrent participation in another therapeutic clinical study;
29) Known hypersensitivity to GS-9674, the metabolites, or formulation excipient;
30) Presence of any condition that could, in the opinion of the investigator, compromise the patient's ability to participate in the study, such as history of substance abuse or a psychiatric (including any patients with a psychiatric hospital admission or emergency room visit in the 2 years prior to screening) or medical condition;
31) Unavailable for follow-up assessment or concern for patient's compliance with the protocol procedures.

Reasons for Screen Failure

	Overall
Screened Subjects	105
Screen Failure Subjects	53/105 (50.5%)
Screen Failure Subjects Who Did Not Meet Eligibility Criteria	51/53 (96.2%)
Inclusion Criterion 04 : Serum ALP > 1.67 x ULN	23/51 (45.1%)
Inclusion Criterion 11 : Subjects with a history of IBD must demonstrate no evidence of active IBD	10/51 (19.6%)
Inclusion Criterion 07 : Screening FibroSURE/FibroTest criterion met	7/51 (13.7%)
Inclusion Criterion 08 : Platelet count criterion met	6/51 (11.8%)
Inclusion Criterion 03 : Diagnosis of PSC based on cholangiogram (MRCP, ERCP, or PTC) within the previous 12 months	5/51 (9.8%)
Exclusion Criterion 06 : Cirrhosis of the liver as defined by any of the criteria in the protocol	4/51 (7.8%)
Inclusion Criterion 17 : Willing and able to comply with all study requirements	4/51 (7.8%)
Exclusion Criterion 03 : Total bilirubin > 2 x ULN	3/51 (5.9%)
Exclusion Criterion 32 : Unavailable for follow-up assessment or concern for subject's compliance with the procedures	3/51 (5.9%)
Inclusion Criterion 09 : Albumin criterion met	3/51 (5.9%)
Inclusion Criterion 10 : Renal function criteria met	3/51 (5.9%)
Exclusion Criterion 04 : INR > 1.2 unless on anticoagulant therapy	2/51 (3.9%)
Exclusion Criterion 01 : Pregnant or lactating females	1/51 (2.0%)
Exclusion Criterion 02 : ALT > 10 x ULN	1/51 (2.0%)
Exclusion Criterion 20 : History of intestinal resection or malabsorptive condition that may limit the absorption of GS-9674	1/51 (2.0%)
Exclusion Criterion 23 : Positive urine drug screen for amphetamines, cocaine, opiates- with exceptions	1/51 (2.0%)
Exclusion Criterion 24 : Unstable cardiovascular disease as defined by any of the criteria in the protocol	1/51 (2.0%)
Exclusion Criterion 27 : Any laboratory abnormality or condition that could adversely affect the safety or impair assessments	1/51 (2.0%)
Inclusion Criterion 02 : Willing and able to give informed consent prior to any study specific procedures being performed	1/51 (2.0%)
Inclusion Criterion 05 : UDCA stable dose or no UDCA use criterion met	1/51 (2.0%)
Screen Failure Subjects Who Met Criteria	2/53 (3.8%)
Reasons for Non-Enrollment of Subjects Who Met Eligibility Criteria	
Study Enrollment Closed	1/2 (50.0%)
Withdrew Consent	1/2 (50.0%)

Supplementary Table 1: Univariate Logistic Regression Assessing Baseline Factors Predicting ALP Response at Week 12

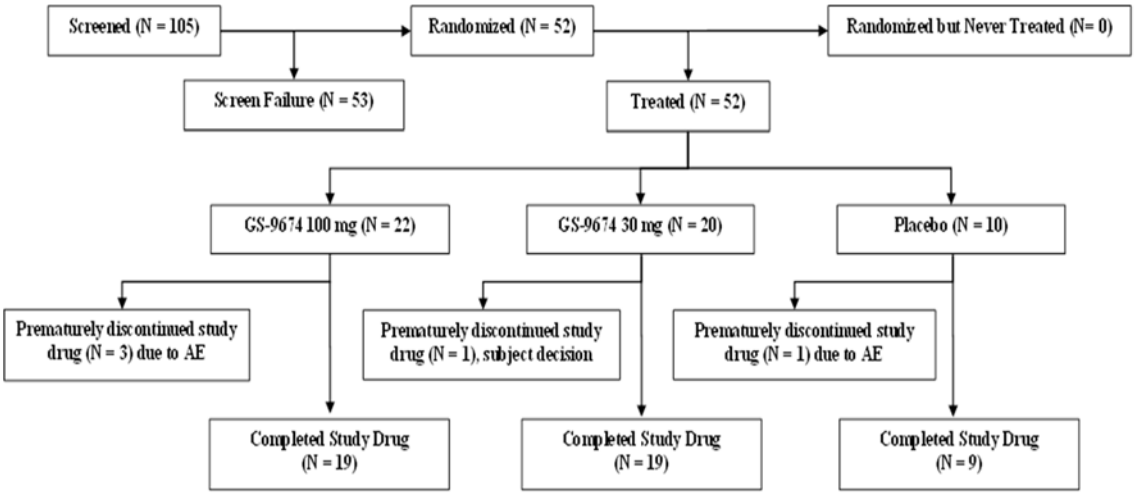
Variable	N	Odds Ratio	95% Confidence Interval	2-Sided P-value
Age, yrs	39	0.97	(0.89, 1.05)	0.42
Male	39	0.55	(0.11, 2.64)	0.46
Weight, kg	39	0.98	(0.93, 1.03)	0.40
Intra- and extra-hepatic duct involvement on MRCP (vs intra-hepatic involvement)	34	0.32	(0.06, 1.75)	0.19
IBD	39	0.72	(0.15, 3.43)	0.68
UDCA use	39	0.83	(0.17, 4.11)	0.82
ALP, per 10-U/L	39	1.01	(0.95, 1.07)	0.81
GGT, per 10-U/L	39	1.01	(1.00, 1.02)	0.17
ALT, per 10-U/L	39	1.03	(0.92, 1.14)	0.66
Total bilirubin, mg/dl	39	2.07	(0.50, 8.58)	0.32
Albumin, g/dl	39	5.50	(0.41, 74.13)	0.20
ELF	39	0.72	(0.27, 1.91)	0.51
FibroTest	38	0.08	(0.00, 21.38)	0.37
Liver stiffness by FibroScan®, kPa	37	0.99	(0.79, 1.24)	0.92
FGF19, per 10-pg/ml	39	1.07	(0.99, 1.16)	0.11
C4, ng/ml	39	0.98	(0.93, 1.03)	0.40
Total bile acids, umol/L	39	1.01	(0.99, 1.03)	0.27
Primary bile acids, ng/ml	39	1.00	(1.00, 1.00)	0.23

ALP response was defined as at least a 25% relative reduction in serum ALP from baseline at week 12. Analysis restricted to cilofexor-treated patients.

Supplementary Table 2: **Treatment-Emergent Laboratory Abnormalities**

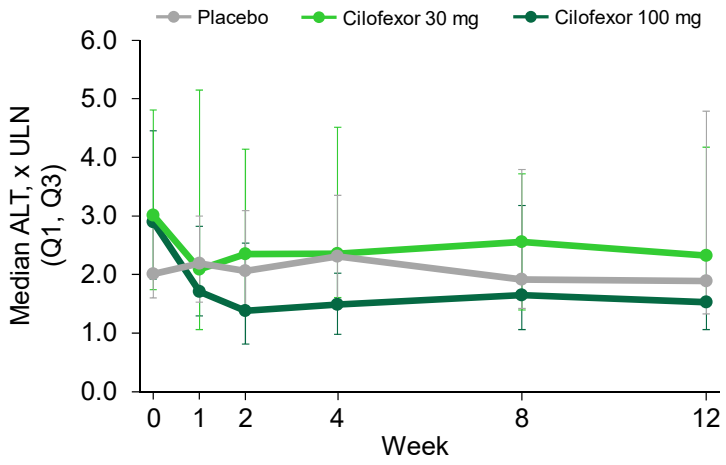
	Cilofexor 100 mg (N = 22)	**Cilofexor 30 mg (N = 20)**	**Cilofexor Pooled (N = 42)**	**Placebo (N = 10)**
Patients with Postbaseline Value	22	20	42	10
Grade 1	5 (22.7%)	5 (25.0%)	10 (23.8%)	1 (10.0%)
Grade 2	8 (36.4%)	7 (35.0%)	15 (35.7%)	6 (60.0%)
Grade 3	6 (27.3%)	4 (20.0%)	10 (23.8%)	3 (30.0%)
Grade 4	1 (4.5%)	1 (5.0%)	2 (4.8%)	0
ALP (U/L) (Increased)	22	20	42	10
Grade 3	2 (9.1%)	1 (5.0%)	3 (7.1%)	1 (10.0%)
ALT (U/L) (Increased)	22	20	42	10
Grade 3	1 (4.5%)	1 (5.0%)	2 (4.8%)	1 (10.0%)
Grade 4	1 (4.5%)	1 (5.0%)	2 (4.8%)	0
AST (U/L) (Increased)	22	20	42	10
Grade 3	2 (9.1%)	4 (20.0%)	6 (14.3%)	1 (10.0%)
Creatinine (mg/dL) (Increased)	22	20	42	10
Grade 3	1 (4.5%)	0	1 (2.4%)	0
GGT (U/L) (Increased)	22	20	42	10
Grade 3	0	0	0	2 (20.0%)
Glucose (mg/dL) (Hyperglycemia)	22	20	42	10
Grade 3	2 (9.1%)	1 (5.0%)	3 (7.1%)	0
Total Cholesterol (mg/dL) (Hypercholesterolemia)	22	20	42	10
Grade 3	1 (4.5%)	0	1 (2.4%)	0

Supplementary Figure 1: Flow Diagram of Study Participants

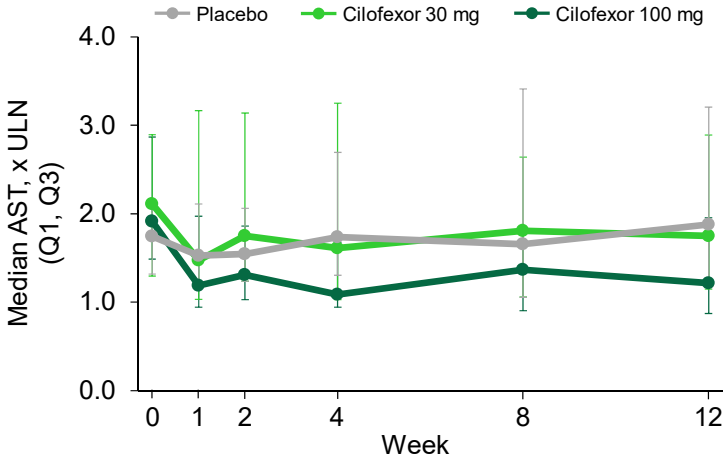


Supplementary Figure 2: Changes in Serum ALT, AST and GGT Relative to the ULN

A)



B)



C)